Elucidating the structure and function of the β-propeller of Erb1 in the context of Nop7-Erb1-Ytm1 complex

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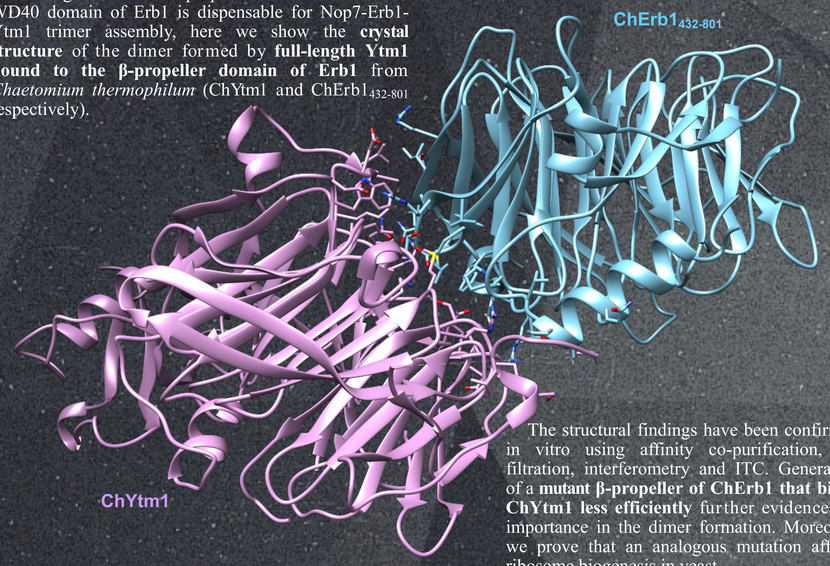
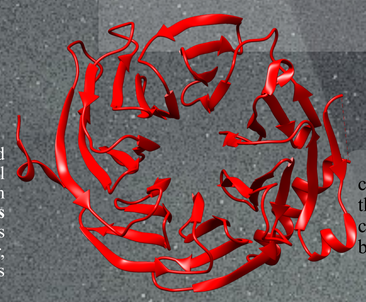
ABSTRACT

Although it has been proposed that the C-terminal WD40 domain of Erb1 is dispensable for Nop7-Erb1-Ytm1 trimer assembly, here we show the **crystal structure** of the dimer formed by **full-length Ytm1 bound to the β-propeller domain of Erb1** from *Chaetomium thermophilum* (ChYtm1 and ChErb1$_{432-801}$ respectively).



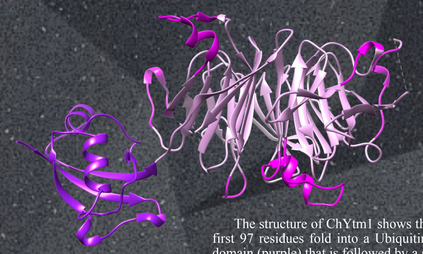
The structural findings have been confirmed in vitro using affinity co-purification, gel filtration, interferometry and ITC. Generation of a **mutant β-propeller of ChErb1 that binds ChYtm1 less efficiently** further evidences its importance in the dimer formation. Moreover, we prove that an analogous mutation affects ribosome biogenesis in yeast.



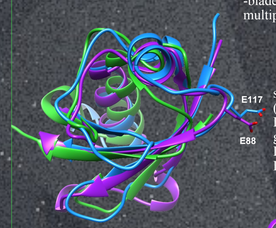
INTRODUCTION

Nop7, Erb1 and **Ytm1** form a stable heterotrimer essential for the maturation of LSU in Eukaryotes[1, 2, 3]. Although the exact role of the complex is not clear, it is required for the processing of 27A$_3$ rRNA[4, 5]. The exact organization of the trimer is unknown but it has been proposed that Erb1 could be the core of the complex as it interacts with both, Nop7 and Ytm1[3]. We have previously solved the structure of the carboxy-terminal domain of Erb1 from yeast and we confirmed that it folded into a seven-bladed β-propeller that could bind RNA in vitro[6]. The apparent lack of importance of this large domain in ribosome biogenesis or in the trimer formation[3] led us to further investigate its function in the cell.

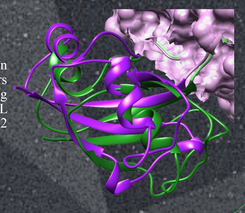
Structure of ChYtm1



The structure of ChYtm1 shows that its first 97 residues fold into a Ubiquitin-like domain (purple) that is followed by a seven -bladed β-propeller (pink) that contains multiple extended loops (magenta).

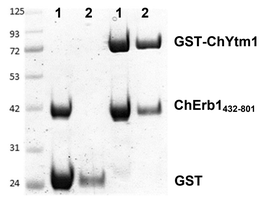


UBL of ChYtm1 (purple) superposes well with ubiquitin (green:UBQ1) and with the UBL of Rsa4 (blue:4WJS). The side chains of glutamic acids from ChYtm1 and Rsa4 that interact with MIDAS of Rea1 are shown.

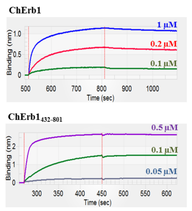


UBL of ChYtm1 presents certain degree of flexibility because it appears in two different orientations depending on the symmetry of the crystals. UBL in purple: P 2$_1$ 2$_1$ 2 and in green: P 6$_5$ 2 2 space groups.

ChYtm1 and ChErb1 interact through their β-propellers



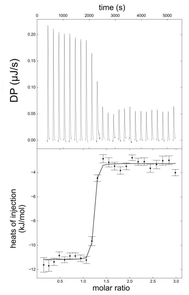
Pull-down showed that 6xHis-tagged ChErb1$_{432-801}$ co-purified with GST-tagged ChYtm1 but not with GST alone on Glutathione Sepharose beads. 1: Input, 2: Elution



Biolayer Interferometry graphs show association and dissociation steps using different concentrations of ChErb1 (top) full-length or ChErb1$_{432-801}$ (bottom) to GST-ChYtm1 immobilized on Anti-GST biosensors. In both cases calculated K_D indicated a good affinity binding

K_D~4x10^{-9} M

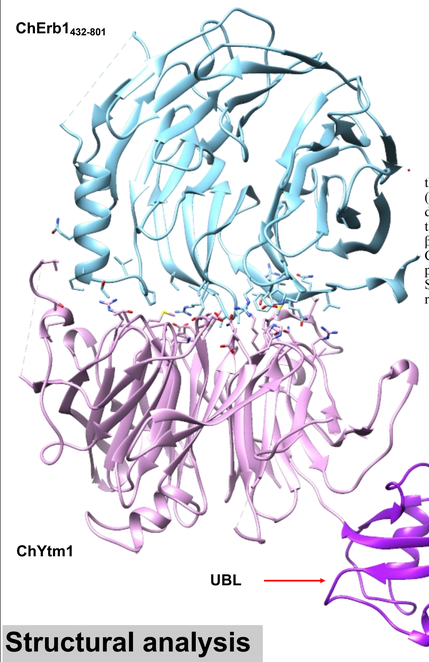
K_D~2.7x10^{-9} M



ITC was done to validate high-affinity binding between ChYtm1 and ChErb1$_{432-801}$. Raw heat of each injection is shown in the upper panel. Curve fitting confirmed that the binding affinity was in low nanomolar range (K_D ~9 nM). Calculated thermodynamic parameters for the ITC experiment are shown in the table.

K_a = 1.088e+08 M^{-1}	**ΔG** = -10.780 kcal/Mol
K_D = 9.193e^{-09} M	**TΔS** = 8.550 kcal/Mol
ΔH = -2.230 kcal/Mol	**ΔS** = 29.165 cal/Mol*K

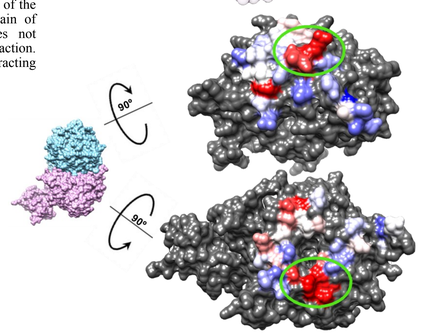
In vitro binding assays



Structural analysis

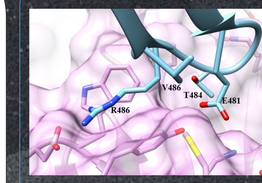
A novel way of interaction between two WD40 domains is observed within the dimer. The central axes of the β-propellers (shown as bars, ChYtm1:pink; ChErb1: blue) form an angle of 55°.

Ribbon representation of the dimer shows that ChYtm1 (pink) binds to the C-terminal domain of ChErb1 (blue) through the top surface of the β-propeller. UBL domain of ChYtm1 (purple) does not participate in the interaction. Side chains of the interacting residues are shown.



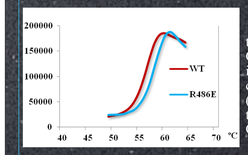
The colored surfaces of ChErb1Ct (top) and ChYtm1 (bottom) show the area that mediates the binding between both molecules. The most variable residues are shown in blue and the most conserved one are depicted in red. Green circles mark the region that was chosen for mutant generation

Mutations of ChErb1Ct



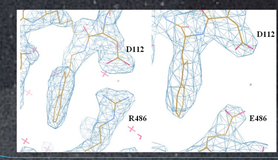
Mutant	S.	SE	K_D (M)
E481R	NO	X	-
E481D	YES	+	5x10^{-9}
T484E	NO	X	-
T484Q	YES	+	3x10^{-9}
R486A	YES	+	n.a.
R486E	YES	-	249x10^{-9}
V488W	YES	+	7x10^{-9}

Detailed view of the most conserved interface of interaction that has been modified in order to alter the binding. The amino acids that have been mutated are labeled. The table summarizes mutations of ChErb1. Soluble variants (YES in S. column) were injected with ChYtm1 in gel filtration (SE column; X: not checked, +: bound, -: no binding) and interaction affinity was measured by interferometry (K_D column).



Thermofluor confirmed that the thermal stability of ChErb1Ct containing R486E in the β-propeller was not compromised and its Tm (blue line) was comparable to the one of wild-type ChErb1$_{432-801}$ (red line).

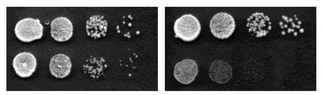
Upon successful **crystallization of ChYtm1/ChErb1$_{432-801}$-R486E** we proved that the β-propeller structure was intact and the only difference between both dimers was the lack of D112-R486 salt bridge (left) that is not formed in the mutant (right).



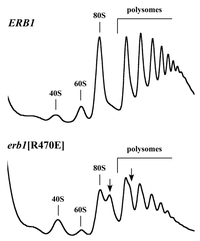
Conclusions

1. The carboxy-terminal domain of **ChErb1** binds to the top face of the β-propeller of **ChYtm1**
2. Fully conserved **salt-bridge** between **R486** from ChErb1 and **D112** from ChYtm1 is required for the binding to occur with high-affinity
3. A mutation that disrupts this salt-bridge in yeast (R470E) negatively **affects growth rate and ribosome biogenesis**
4. Ytm1 **cannot stably associate** with pre-ribosomes when Erb1 carries R470E mutation on its C-terminus
5. The β-propeller of Erb1 **is involved** in the assembly of Nop7-Erb1-Ytm1 trimer and 60S synthesis.

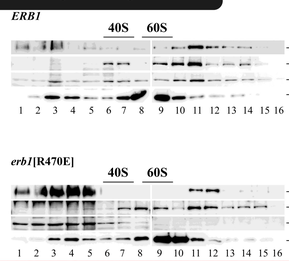
Erb1[R470E] affects 60S assmebly in yeast



Erb1 or Erb1[R470E] were used to complement Δ*erb1* strain. *Erb1*[R470E] allele affected significantly growth of *S. cerevisiae* in a temperature-dependent manner. Thus, while growth rate is mildly reduced at 30 ºC, it is severely affected at 37 ºC



Compared to wild-type strain, *erb1*[R470E] cells showed a strong decrease in the levels of free 60S r-subunits, an increase in the levels of free 40S r-subunits, and overall decrease in the 80S peak and in polysomes. Most importantly, there was an accumulation of half-mer polysomes only in *erb1*[R470E] cells (arrows).



Fractionation of r-subunits on sucrose gradients. In wild-type strain of yeast little amount of Erb1, Nop7 or Ytm1 could be detected at the top of the gradient, suggesting that most proteins are stably associated with pre-60S. In contrast, less Erb1[R470E] protein was detected in high MW fractions. A large amount of Erb1[R470E] was present at the top of the gradient. Whereas Nop7 distribution was similar in both cases, high amount of Ytm1 was found in low MW fractions and no Ytm1 was associated with pre60S indicating that the incorporation of Ytm1 into pre-ribosome is impaired in the context of Erb1-R470E.

References

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